FOREIGN BODY REACTIONS INDUCED BY FUNGI IRRADIATED IN SPACE

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Introduction: The closed environment of spacecraft during extended journeys and the stresses of spaceflight create conditions favorable to induce change in microorganisms. Four fungal species were selected for exposure in space and were returned to earth for postflight evaluations. Phenotypes of Chaetomium globosum Kunze, Rhodotorula rubra (Demme) Lodder, Saccharomyces cerevisiae Hansen, and Trichophyton terrestre Durie et Frey were selected from the Apollo 16 Microbial Ecology Evaluation Device (MEED) after exposure to specific spaceflight parameters (Volz, 1975). Mycological studies in space have been numerous, beginning with high altitude balloon flights that expanded defined experiments beyond the atmosphere of earth (Dublin and Volz, 1973). Previous postflight evaluations of the spaceflight fungal phenotypes currently under study demonstrated variation at the cellular level (Dublin, et al., 1974; Sawyer, et al., 1975; Volz, 1975; Volz and Dublin, 1973; Volz, et al., 1974; Wurzburger and Volz, 1976). Changes in survival rates and phenotype counts, nutritional requirements, cellular response to drugs, growth rates, mycelial growth in the presence of salivary peroxidase activity, rate of human hair deterioration, and change in whole cell phospholipid contents were attributed to spaceflight exposure. The Apollo MEED postflight studies continue to further identify change in living systems initially exposed to parameters of space. Because of the biomedical changes, the current study was designed to investigate foreign body reactions in animals caused by fungi after exposure to parameters of space.

Materials and Methods: Ascospores of Chaetomium globosum ATCC 6205, conidia of Trichophyton terrestre x285, and vegetative yeast cells of Saccharomyces cerevisiae y2439 and Rhodotorula rubra y1592 were housed in distilled water or dry in 0.05 ml volume square cuvettes within the MEED spaceflight hardware. Each cuvette contained a quartz window and a series of filters to regulate the ultraviolet light (UV) wavelength and intensity at exposure in space (Taylor, 1970). The MEED was deployed at a 90° angle to the sun for 10 min + 7 sec during the transearth Extra Vehicular Activity (EVA) of Apollo 16 (Volz, 1975). Fungi in the flight hardware were exposed to 254, 280, and 300 nanometers (nm) UV light at various energy levels during deployment and attachment of the MEED flight hardware on the television campole extension and Command Module hatch. After exposure, the flight hardware was stowed and returned to the lab at

splashdown (Volz, 1974). Fungal cells housed in the flight hardware were placed on Sabouraud maltose agar and initially studied for survival capabilities according to exposure levels in space (Volz and Dublin, 1973).

Phenotypes for the present study were obtained from viable cells collected in postflight studies. The phenotypes were selected in relation to wild type by colony morphology, growth rate, growth density, colony texture, and variation in sporulation and pigmentation (Volz, 1974). From 10 to 15 phenotypes of each fungal species in addition to the wild type or ground control were used in this study.

Swiss Flow DUB/KR Mice and Golden Syrian Hamsters from a random breed closed colony were pretreated with 20 mg/ml daily inoculations of hydrocortisone acetate suspension for one week prior to the introduction of the fungal test organisms to repress the defense system of the animals. Pretreated animals and control animals not receiving hydrocortisone acetate were inoculated with suspensions of the test fungi.

Mice received 0.2 ml cell suspension in 0.9% saline intraperitoneally, in the tail vein, and the epidermis while hamsters were injected with 0.9 ml cell suspension in the cheek pouch and gum area at a cell concentration of $1 \times 10^6 - 10^8$ cells per ml. Before inoculation, hamsters were anesthetized with 7.5 mg/ml sodium pentobarbital using 1 ml (50 mg/ml) per 100 g weight of animal. Animals remained anesthetized for a sufficient time to withdraw the buccal pouch and make the inoculations. Histological studies were made when changes in the normal behavioral activity in the animals were noted, about 4 to 6 weeks after inoculation. Approximately 0.25 g material from isolated lesions were inoculated on Sabouraud maltose agar for fungal recovery. Other lesion tissue was placed in Bouin's fixative, dehydrated in a alcohol series, Feulgen stained, embedded in Tissuemat, sectioned, and described. Replicates of 5 animals were used for each test phenotype and control.

Results: The spaceflight exposures received by the phenotypes are presented in Table 1. Greatest morphological diversification in phenotypes compared with the wild type or parent strain was the principal method in selection of test organisms for the current evaluations.

Viable cells were recovered from mouse tissue streaked on agar plates as shown in Table 2. Mouse lesions induced by Chaetomium globosum involved the subcutaneous area. The cellular response was lymphocytic. Ascospores and hyphae were seen external to the muscle sheath and a small wall of lymphocytes generally separated the fungal cells from the muscle sheath. Muscle tissue was not affected, but ascospores were seen between muscle sheaths.

Other involvements of C. globosum in mice included the liver, kidney, and spleen (Fig. 1). An abcess lesion in the liver originated in a central vein and involved an entire lobe. Liver involvements were circumscribed nonencapsulated areas of inflammation with a central necrotic core. Destruction of the liver parenchyma was

present and the mononuclear inflammatory cells were primarily lymphocytes. The central core of lesions contained tissue debris and liver cells in various stages of degeneration.

Trichophyton terrestre phenotypes and T. terrestre wild type produced diffuse subcutaneous lesions. A rudimentary wall around the areas, caused by a fibrocytic reaction, was present. The central area of the lesion appeared granulomatous, with necrotic cells and fungal conidia present. There were many polymorphonuclear cells in the center near the cellular debris and conidia.

Subcutaneous mouse lesions of Saccharomyces cerevisiae were large and fairly well circumscribed. They affected the subcutaneous region and did not affect the overlying skin. No ulceration occurred but lesions were intense inflammatory reactions. Centers were necrotic and infiltrates were a mixture of polymorphonuclear cells surrounded by monocytes. Budding yeast cells were very abundant. Lesions were well circumscribed and encapsulated. A large quantity of cellular infiltrate, including many lymphocytes and marcophages were present. This was an intense necrotic reaction with a large amount of cellular debris and some calcium deposits in response to the inflammation. In the periphery of the necrotic areas proliferating fibrocytes were found.

Liver lesions initiated by S. cerevisiae wild type and phenotypes were fairly well walled off with fibrocytes and macrophages (Fig. 2). It was an acute reaction, and liver tissue involved was necrotic. Older liver lesions were granulomatous and contained a mononuclear infiltrate at the periphery. In lesions much debris was present and no cellular detail was evident. Lesions originated near central veins.

With Rhodotorula rubra wild type and pheontypes a mild dermal reaction was seen in mice that was not walled off. However, a rudimentary wall was present since much fibrous proliferation took place which did not circumscribe the diffuse lesion (Fig. 3). Many polymorphonuclear cells were evident in the area of yeast cells. Some degenerating muscle tissue was present in lesions. Internal organs were not infected. A significantly higher number of viable cells were recovered from skin lesions of S. cerevisiae phenotypes compared with lesions induced by S. cerevisiae wild type control (Table 2).

Cortisone pretreated hamsters inoculated with the phenotypes elicited foreign body reactions. The invaded areas involved a large tissue area adjacent to the central lesion. Cellular response in the hamsters was similar to that found in mice with the respective fungal phenotypes. In general, S. cerevisiae in hamsters produced soft, highly inflamed, diffuse buccal pouch lesions with proliferation of the yeast cells. Rhodotorula rubra induced lesions were soft, mildly inflamed, and diffuse. Trichophyton terrestre initiated only small nodular lesions at the site of inoculation and C. globosum induced lesions were localized, small, hard, and nodular. Pretreated animals and animals with no hydrocortisone acetate produced no variation in tissue response.

Discussion: Varying degrees of inflammatory responses were noted with the test fungi. Order of reactivity observed in the mice and hamsters from the most severe to the least was Saccharomyces, Rhodotorula, Chaetomium, and Trichophyton. Phenotypes of S. cerevisiae gave very diffuse and very intense reactions sometimes to the point of being a true abcess. The S. cerevisiae cells would often proliferate in the host. The lesions were partially circumscribed and not encapsulated although fibrosis was evident. The cellular response was mainly mononuclear with macrophages and polymorphs usually in the area. There was seldom a central core of necrosis, although often scattered pycnotic nuclei were seen as well as a large amount of cellular debris. The gross lesion was relatively soft as compared to the hard nodular lesion of C. globosum, and large amounts of pus were always present.

The cellular and gross appearance of the response to R. rubra was similar to that of S. cerevisiae but less severe. Similarly with Saccharomyces, the Rhodotorula cellular response was mainly mononuclear with polymorphs and macrophages in the area. The lesions were diffuse but were more contained than those of S. cerevisiae. More fibrous proliferation and a more definite area of necrosis were present. In the lesion area many pycnotic nuclei and some cellular debris were commonly seen. The gross lesions of R. rubra were very similar to the gross lesions of Saccharomyces except slightly smaller and slightly less intense in adjacent tissue.

The cellular response to C. globosum was a circumscribed well defined area of approximately 95% lymphocytes that was well encapsulated with a thin fibrous sheath. Proliferation of the cells was never observed. Cellular debris, some pycnotic nuclei, and cells in various stages of degeneration were present inside the circumscribed area. The gross lesion was small, hard, and nodular, and the area adjacent to the lesion was not inflamed.

Trichophyton terrestre phenotypes were the least reactive, and seldom lesions were produced to combat their presence. When a lesion did form, it was moderately diffuse and there was a pronounced fibrocytic reaction that did not completely encapsulate the area. Necrotic cells and cellular debris were in the lesion area.

Variations between phenotypes within the same species were not as pronounced as foreign body reactions between the selected genera. Phenotypes of Saccharomyces cerevisiae showed more reactivity than other test fungi. In addition, more viable cells were recovered from the dermal lesions in mice induced by phenotypes than from lesions initiated by the wild type.

Summary: The cellular response to Chaetomium globosum, Rhodotorula rubra, Saccharomyces cerevisiae, Trichophyton terrestre and their spaceflight phenotypes was a foreign body reaction. Response variation in mice and hamsters was greater between genera and less evident between phenotypes of the same species. Cells of S. cerevisiae exposed to spaceflight parameters retained a higher recovery rate in dermal lesions compared with cells isolated from lesions induced by S. cerevisiae ground control.

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Table 1

Exposures in space of test fungi included in the foreign body reaction studies

Organism	UV wavelength	Energy level
Chaetomium globosum test*	full light of space	$1 - 5 \times 10^5$ ergs
C. globosum wild type	0	0
Trichophyton terrestre test	280 nm	$2 - 9 \times 10^4$
T. terrestre wild type	0	0
Saccharomyces cerevisiae test	254 - 300 nm	$7 \times 10^3 - 7 \times 10^4$
S. cerevisiae wild type	0	0
Rhodotorula rubra test	280 nm - full light	$2 \times 10^4 - 2 \times 10^5$
R. rubra wild type	0	0

*Data presents the range of exposure levels in space for each species, compiled from measured irradiations for each test phenotype.

Table 2

Average number of colony forming units per plate obtained from wild type and phenotypes recovered from mouse tissue.

Wild type	skin	liver	kidney	spleen
Saccharomyces cerevisiae	9	6	2	9
Rhodotorula rubra	1	0	0	1
Trichophyton terrestre	0	0	0	0
Chaetomium globosum	20	7	9	12
Spaceflight phenotypes*				
Saccharomyces cerevisiae	168	7	7	5
Rhodotorula rubra	4	0	0	0
Trichophyton terrestre	0	0	0	0
Chaetomium globosum	27	2	7	3

*Average of 10 phenotypes for each species.

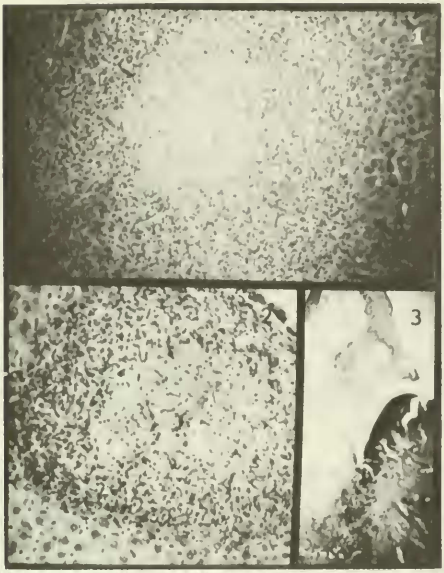

Figure legends

Fig. 1. Chaetomium globosum induced lesion in mouse liver (x 170).

Fig. 2. Saccharomyces cerevisiae initiated mouse liver lesion (x 220).

Fig. 3. Foreign body reaction of Rhodotorula rubra in mouse dermis (x 90).

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